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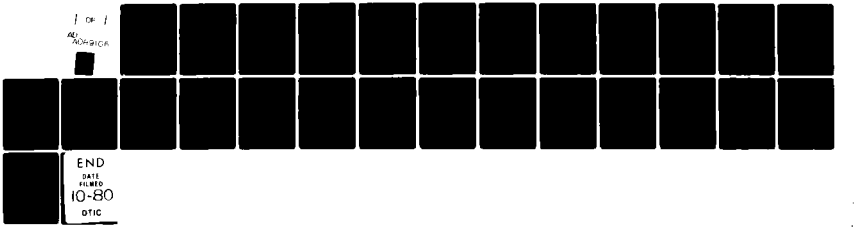

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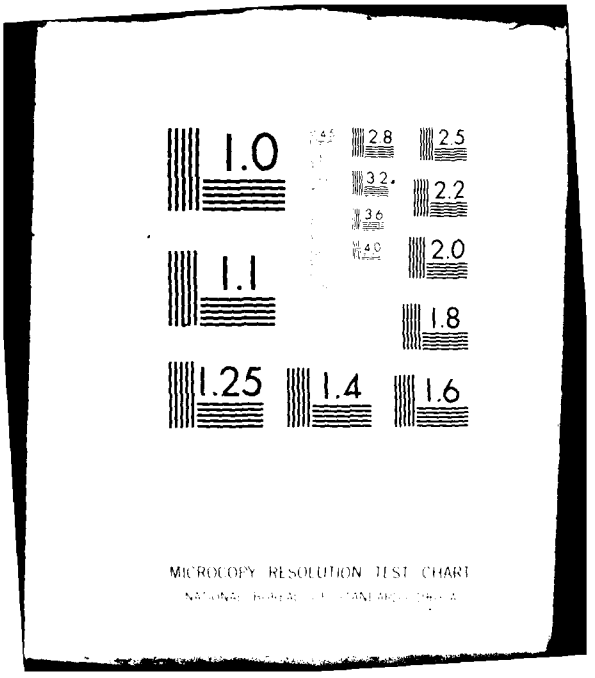
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Report No. 15

Annual Progress Report

BIOCHEMICAL CHANGES IN TISSUES DURING INFECTION ILLNESS: METABOLIC CONSEQUENCES OF INTERACTIONS BETWEEN INFECTIOUS ILLNESS AND FORCED EXERCISE

(12)

by

Robert L. Squibb, Ph. D.

September 1980

(For the period 1 July 1979 to 30 June 1980)

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U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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Infectious illness, forced exercise and energy source were studied in one, two or three-way interactions. Both weanling male rats and day-old cockerel chicks were used to observe species differences. Challenges with infectious illness, e.g., S. typhimurium for rats and avian tuberculosis for chicks, were considered low level. Maximum forced exercise (running wheels) period was a 2-hour session at 10 rpm. Nutritional sub-plots were included which permitted comparisons of glucose, fructose or sucrose as the major source of dietary energy. —
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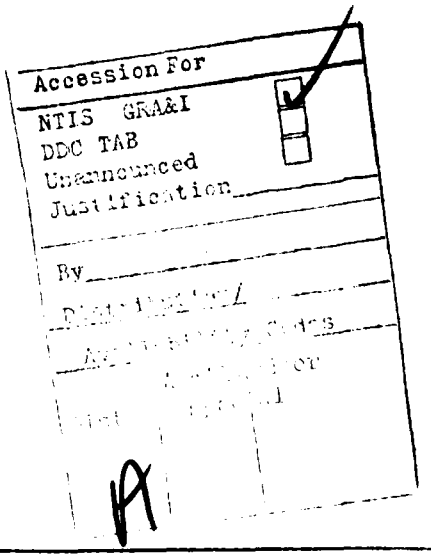
Statistical analyses of the data indicated that infection and forced exercise effects were specific with little interaction. Not only were liver parameters affected but also protein synthesis in the heart; these effects were more pronounced when fructose was fed. Liver glucose and glycogen levels were slightly lower in control and infected rats forced to run 2 hours than in rats allowed to exercise voluntarily for the same length of time. Of the biochemical data observed, liver glycogen appeared to have the most immediate response to infection and/or exercise. A reduction in hepatic glycogen generally correlated with a decrease in triglycerides in fat pads and an increase in blood transport.

Chicks fed large quantities of fructose required an adaptation period; those fed glucose did not. Neither fructokinase nor aldolase were involved in this phenomenon. In the presence of avian TB, body and liver weights were depressed and glucose, glycogen and lipid stores variably reduced. A single 2-hour bout of forced exercise in noninfected chicks depleted liver glucose and glycogen without affecting lipid levels.

S. typhimurium significantly reduced triglycerides in plasma, liver and fat pads while plasma and liver cholesterol were increased; cholesterol and free fatty acids were decreased in fat pads. Plasma betahydroxybutyrate (BOHB) and total ketone bodies were repressed by the endotoxin response of S. typhimurium while acetoacetate was not affected. In fasted rats (simulating the anorexia of infectious illness) fat pad size decreased 44% while a 2-hour forced exercise period resulted in only a 3% decrease. In fed rats, infection decreased and exercise increased fat pad size 19 and 7%, respectively. Exercise increased BOHB 13-fold in controls and only 7-fold in infected rats. The insulin:glucagon ratio was decreased 65% by both fasting and exercise. Our level of disease challenge had no apparent effect on this ratio.

An overview of our data on sucrose vs fructose as the major source of dietary energy during infectious illness and forced exercise tends to indict the nutritional worth of fructose. Basis for this statement: 1) increased mortality in infected animals; 2) adverse effects on protein synthesis in the rat heart; and 3) depression of liver glycogen stores in both rats and chicks. This is of military importance since fructose now makes up a significant part of the human diet.



SUMMARY

This annual report summarizes the results of some 26 experiments wherein infectious illness, forced exercise and energy source were studied in one, two or three-way interactions. Both weanling male rats and day-old cockerel chicks were used to observe species differences. Challenges with infectious illness, e.g., S. typhimurium for rats and avian tuberculosis for chicks, were considered low level. Maximum forced exercise (running wheels) period was a 2-hour session at 10 rpm. Nutritional sub-plots were included which permitted comparisons of glucose, fructose or sucrose as the major source of dietary energy.

Statistical analyses of the data indicated that infection and forced exercise effects were specific with little interaction. Not only were liver parameters affected but also protein synthesis in the heart; these effects were more pronounced when fructose was fed. Liver glucose and glycogen levels were slightly lower in control and infected rats forced to run 2 hours than in rats allowed to exercise voluntarily for the same length of time. Of the biochemical data observed, liver glycogen appeared to have the most immediate response to infection and/or exercise. A reduction in hepatic glycogen generally correlated with a decrease in triglycerides in fat pads and an increase in blood transport.

Chicks fed large quantities of fructose required an adaptation period; those fed glucose did not. Neither fructokinase nor aldolase were involved in this phenomenon. In the presence of avian TB, body and liver weights were depressed and glucose, glycogen and lipid stores variably reduced. A single 2-hour bout of forced exercise in noninfected chicks depleted liver glucose and glycogen without affecting lipid levels.

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An overview of our data on sucrose vs fructose as the major source of dietary energy during infectious illness and forced exercise tends to indict the nutritional worth of fructose. Basis for this statement: 1) increased mortality in infected animals; 2) adverse effects on protein synthesis in the rat heart; and 3) depression of liver glycogen stores in both rats and chicks. This is of military significance since fructose now makes up a large part of the human diet.

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INTRODUCTION

We commented in our 1980 application for support of research related to biochemical changes in tissues during infectious illness x forced exercise x energy source that our asking budget of approximately $22,000 was extremely modest. Nevertheless, we expected to make a significant contribution(s) to the understanding of biointeractions between infectious illness x exercise x energy source because several advanced graduate students were interested in working in this area. Individual contributions of each of these students will be noted in their forthcoming technical publications.

The following is a list of the contributors to this annual report:

Balentine, Douglas (starting Ph.D.)
Chaudhuri, Minu (finished Ph.D., 1980)
Douglas, Gary (mid stage Ph.D.)
Gunnar-Ilback, Nils (mid stage Ph.D.)
Kenler, Hallis (finished Ph.D., 1980)
Rudolph, Herbert (finished M.S., 1980)

In addition, there was a cooperative effort with the Rutgers Dept. of Nutrition.

The very modest funding of our contract served, and we hope will continue to serve, as a catalyst for our research. The participation of graduate students has allowed us to broaden our approach to the problem under investigation. As a consequence, we have been able to include two different species and relate the effects of different sources of dietary energy to the problem. These additions do not deviate but add to our initial objectives. Equally important, the present report presents data that show that the recognition and understanding of the effects of dietary energy sources are vital to the interpretation of results obtained in this complex field.

As may be appreciated, we have amassed a considerable quantity of data. As a consequence, we are only able to present selected portions of that data. The reader should understand that the complete presentations can be found in the theses presented to the Rutgers Graduate School. In addition, the theses research will be submitted to technical journals as rapidly as possible.

COMMENTS ON THE EXPERIMENTAL DESIGNS USED

An infinite selection of experimental designs can be used in studies on the interaction of infectious illness with exercise and diet. In our ongoing research we have dealt principally with low level infections and have sampled tissues during the incubation stage of the infection. This is in contrast to our colleagues at USAMRIID, Fort Detrick, who employ massive doses yielding up to 100% mortality.

The immediate questions posed in our initial studies of the above interactions were: How much forced exercise to employ? What level of disease involvement? What time should we inoculate, exercise and sacrifice?

Our first approach has been to employ the stress at its lowest level since we would necessarily have to use small numbers of animals to equate to our forced running equipment and our labor supply at time of sacrifice. With regard to the latter, we have followed previous procedures which call for the sacrifice periods to be completed in less than one hour in order to avoid circadian confounding.

Dr. Morris Solotorovsky, Pathogenic Bacteriologist at Rutgers, prepares the S. typhimurium inocula; standardization is by densitometer readings and susceptible weanling rats are used to check results. A dose of 0.2 ml/rat, i.p. results in enlarged spleens and livers and the characteristic clinical symptoms of S. typhimurium. Dr. Solotorovsky also confirms the presence of the organism and the fact that there is no contamination of the control animals.

Selection of a single 2-hour forced exercise session of a naive subject was to make certain we were not confounding results with prior conditioning, which is another important area we are investigating.

As to diet, we felt that dietary source of energy could have a distinct bearing on the proposed interactions. Such data are also of interest to the military, especially in combination with forced exercise and infectious illness.

I.

EFFECT OF DIETARY ENERGY SOURCE AND FORCED EXERCISE ON METABOLISM OF CHICKS

These trials were run prior to subsequent studies on forced exercise x infectious illness in order to gain an understanding of the need to consider dietary energy sources in future experimental designs. Three replicate trials were run sequentially, with numbers of animals limited to the capacity of our "forced running" equipment.

The effects of forced exercise were compared between groups of chicks fed diets containing either 50% fructose, glucose or sucrose. In each trial the chicks were subjected to a forced exercise session of 30 min the first day, 45 min the second day and 60 min on the third day and then were sacrificed.

Body weights, liver weights and liver/body weight ratios were lowest in chicks fed the fructose diet. (table 1). Forced exercise depressed body weights and reduced total quantities of fructose, glucose and glycogen in the liver (table 1). Under the conditions of these trials, forced exercise did not cause any significant changes in the total quantities of liver lipids (table 2). The fructose dietary group had higher amounts of liver and plasma fructose but lower quantities of hepatic glucose, glycogen and lipid components.

These results indicate 1) a metabolic disorder due to high fructose intake; 2) that dietary energy source must be defined for studies of interactions with infectious disease; and 3) that further experimentation related to this contract should consider dietary energy source as another treatment having the potential of interacting both synergistically and antagonistically, thus affecting interpretation of the data.

Table 1

Body and liver weights and total quantities of liver fructose, glucose and glycogen in chicks fed varying sources of energy and subjected to forced exercise

Diet	Forced exercise 1/	Body weight	Liver weight	Liver wt. / Body wt.	Fructose	Glucose	Glycogen
		g	g	%	mg/total liver		
Trial 1							
Fructose	No	95.7	4.5	4.7	0.4	6.9	41.6
"	Yes	85.3	4.0	4.7	0.2	2.8	12.7
Glucose	No	99.3	5.1	5.1	0.2	7.6	196.0
"	Yes	93.3	4.6	5.0	0.1	1.3	1.1
Sucrose	No	104.7	5.2	4.9	0.2	7.0	112.9
"	Yes	97.3	4.8	4.9	0.1	2.6	11.0
ANOVA, F value:							
Diet		7.1**	8.5**	3.9*	1.9	0.0	0.4
Exercise		12.0**	7.9*	0.4	5.2*	11.4**	2.5
D x E		0.3	0.0	0.2	0.2	0.2	0.6
Trial 2							
Fructose	No	106.3	4.5	4.2	0.4	6.1	50.3
"	Yes	95.7	4.4	4.6	0.1	3.5	34.0
Glucose	No	136.7	7.2	5.3	0.3	8.3	107.3
"	Yes	127.7	7.4	5.8	0.1	2.9	39.3
Sucrose	No	143.7	6.3	4.4	0.2	9.2	397.6
"	Yes	129.0	6.5	5.0	0.2	4.5	109.2
ANOVA, F value:							
Diet		26.7**	131.6**	12.0**	0.8	1.3	2.3
Exercise		7.0*	0.3	6.4*	5.4*	16.6**	2.3
D x E		0.2	0.2	0.1	1.0	0.7	1.0
Trial 3							
Fructose	No	131.3	6.5	5.0	0.7	7.4	85.7
"	Yes	124.3	6.3	5.1	0.5	4.6	76.1
Glucose	No	168.3	8.4	5.1	1.0	14.5	310.6
"	Yes	159.3	8.2	5.2	0.7	6.4	245.2
Sucrose	No	168.3	6.3	4.1	1.1	14.2	243.7
"	Yes	164.3	7.1	4.3	0.3	2.6	10.0
ANOVA, F value:							
Diet		10.5**	13.9**	4.2*	2.0	2.9	3.5
Exercise		0.3	0.0	0.3	15.4**	24.7**	2.4
D x E		0.0	0.2	0.0	3.2	2.9	1.6

1/ Within each trial, 30 min forced exercise (running wheels) on day 1, 45 min on day 2 and 60 min day 3 and then sacrificed

* $P < 0.05$; ** $p < 0.01$

Table 2

Total quantities of lipid components in livers of chicks fed varying energy sources and subjected to forced exercise

Diet	Forced exercise[1]	MG[2]	DG[2]	TG[2]	FFA[2]	Chol[2]	Choi. esters[2]
		mg/total liver					
Trial 1							
Fructose	No	5.7	9.1	20.1	16.0	12.8	12.4
"	Yes	4.9	7.0	13.4	11.9	10.6	10.4
Glucose	No	7.1	11.8	25.4	18.2	14.4	14.9
"	Yes	6.9	13.2	25.3	18.9	13.4	13.7
Sucrose	No	8.4	11.9	25.6	18.2	14.8	14.5
"	Yes	6.4	8.9	20.2	15.5	13.0	11.7
ANOVA, F value:							
Diet		2.4	4.6*	7.8**	3.7	2.5	7.1**
Exercise		1.6	1.0	5.0*	2.1	3.4	9.7**
D x E		0.4	1.2	1.2	1.1	0.2	0.6
Trial 2							
Fructose	No	7.4	7.7	17.0	17.1	13.0	10.2
"	Yes	9.1	8.7	16.0	15.2	13.9	11.1
Glucose	No	8.5	21.1	43.1	28.0	19.4	20.5
"	Yes	7.6	19.3	53.2	27.2	19.8	23.5
Sucrose	No	9.2	15.2	41.2	26.8	18.2	20.8
"	Yes	7.8	15.8	36.8	26.3	17.9	15.9
ANOVA, F value:							
Diet		0.2	19.2**	25.8**	11.6**	31.4**	36.7**
Exercise		0.1	0.0	0.2	0.2	0.3	0.1
D x E		0.8	0.3	1.4	0.0	0.3	4.6*
Trial 3							
Fructose	No	13.7	15.9	30.1	24.3	18.2	14.4
"	Yes	10.9	14.4	26.9	22.5	20.0	14.4
Glucose	No	15.5	21.3	52.1	36.0	27.0	18.5
"	Yes	19.4	21.9	47.0	42.5	28.0	25.4
Sucrose	No	8.6	12.8	27.4	26.4	19.7	18.8
"	Yes	10.9	22.7	32.3	41.9	27.3	20.1
ANOVA, F value:							
Diet		7.7**	6.1**	6.8**	10.5**	15.5**	11.7**
Exercise		0.5	3.8	0.0	5.5*	8.0*	0.0
D x E		1.5	5.3*	1.1	3.0	2.9	0.1

1/ Within each trial, 30 min forced exercise (running wheels) on day 1, 45 min on day 2 and 60 min day 3 and then sacrificed

2/ MG = monoglycerides; DG = diglycerides; TG=triglycerides; FFA=free fatty acids; Chol = cholesterol

*P<0.05 **P<0.01

Table 3

Plasma glucose and fructose content in chicks fed varying sources of energy and subjected to forced exercise

Diet	Forced exercise 1/	Fructose	Glucose
		mg/100 ml plasma	
Trial 1			
Fructose	No	9.3	66.1
"	Yes	5.3	61.0
Glucose	No	4.0	63.2
"	Yes	3.3	54.5
Sucrose	No	8.7	67.6
"	Yes	5.3	63.2
ANOVA, F value:			
Diet		3.8*	0.9
Exercise		5.0*	2.2
D x E		0.7	0.1
Trial 2			
Fructose	No	68.7	77.0
"	Yes	17.3	68.3
Glucose	No	5.3	62.5
"	Yes	6.7	53.1
Sucrose	No	4.3	52.3
"	Yes	8.7	74.9
ANOVA, F value:			
Diet		33.3**	1.5
Exercise		15.2**	0.0
D x E		17.8**	2.3
Trial 3			
Fructose	No	9.3	67.6
"	Yes	9.3	79.2
Glucose	No	4.7	66.1
"	Yes	4.7	67.6
Sucrose	No	8.0	72.7
"	Yes	6.0	66.1
ANOVA, F value:			
Diet		7.7**	0.9
Exercise		0.5	0.3
D x E		0.5	1.8

1/ Within each trial, 30 min forced exercise (running wheels) on day 1, 45 min on day 2 and 60 min day 3 and then sacrificed

* $P < 0.05$; ** $P < 0.01$

II.

POTENTIAL SIGNIFICANCE OF CARNOSINE IN THE INFECTED TISSUES OF RATS AND CHICKS

In a cooperative study with the Rutgers Nutrition Department, concentrations of the imidazole-containing compounds anserine, carnosine and free histidine were determined in the muscle tissue of male chicks inoculated with low and high levels of avian tuberculosis and in weanling rats infected with Salmonella typhimurium. Both types of infection resulted in a decrease in carnosine (table 4). concentrations. On the other hand, anserine concentrations were not affected by either the low or high levels of infection. Tissue free histidine increased significantly due to high levels of infection, with no apparent response to low infection levels. The results are consistent with an hypothesis that carnosine acts as a tissue reservoir for histidine, ultimately serving as a precursor of histamine.

Table 4. Anserine, carnosine and histidine concentrations in pectoral muscle of young cockerels infected with Mycobacterium avium (tuberculosis) and in leg muscle of young rats infected with S. typhimurium.

Tissue	State of infection	Anserine	Carnosine	Free-histidine
			μ moles/g wet tissue	
Cockerels 21 days after inoculation				
Pectoral muscle	None	16.0 ± 1.2*	14.7 ± 1.4[a]	0.15 ± 0.07
Pectoral muscle	Infected	17.3 ± 1.4	8.6 ± 2.0[a]	0.13 ± 0.08
Rats one day after inoculation				
Leg muscle	None	2.6 ± 0.28	7.1 ± 0.62	0.64 ± 0.07
Leg muscle	Infected	3.0 ± 0.92	6.8 ± 0.96	0.79 ± 0.21
Rats 9 days after inoculation				
Leg muscle	None	1.2 ± 0.23	4.0 ± 0.41[a]	1.21 ± 0.17
Leg muscle	Infected	1.0 ± 0.09	2.6 ± 0.38[a]	1.15 ± 0.24

*Mean ± SE for 7 cockerels or rats per treatment

[a]Difference between infected and noninfected values for the same tissue is significant at $P < 0.01$ (Student's t test)

III.

UPTAKE AND METABOLISM OF FRUCTOSE DURING DIETARY LOADING OF CHICKS INFECTED WITH AVIAN TUBERCULOSIS

This section is a summary of some 10 experiments. Only a sample table is included here; the remainder of the data appear in a Ph.D. thesis of Minu Chadhuri, Rutgers University, 1980.

The results of these experiments indicate that fructose required a period of dietary adaptation before it was fully utilized by the chick. This period of adaptation was marked by clinical changes characterized by poor feathering and watery feces, and by depleted energy stores and high levels of plasma fructose. The enzymes fructokinase and aidolase apparently were not involved in the required period of adaptation since there were no changes in the levels of fructose-1-P in the liver.

The presence of fructose in the diet either as sucrose or as invert sugar did not influence the adaptation response.

Control chicks and those infected with avian tuberculosis and fed high levels of fructose showed greater depletion of metabolic energy stores than those fed glucose as the principal carbohydrate. A general lowering of energy stores correlated with a low degree of TB involvement. An interaction between the sugar used and TB with respect to the total triglyceride and glycogen levels was observed with the high degree of TB involvement.

Over a 4 hour period post loading with fructose or glucose, there was an increased synthesis of triglycerides and glycogen from both sugars. Fructose initially produced lower levels of glycogen than glucose, but after the fructose levels started to decline in the plasma more glycogen was observed in the livers of the chicks loaded with fructose than those loaded with glucose.

Tuberculosis infection equally lowered the levels of triglycerides in the livers of chicks loaded with either fructose or glucose. However, for glycogen the depression in liver levels was larger for chicks loaded with fructose than for those loaded with glucose.

Table 5. Body and liver weights, liver glucose and glycogen, and plasma glucose and fructose values of chicks loaded with glucose or fructose and infected with a high level of tuberculosis

Disease	Sugar	Body wgt.	Liver wgt.	Liver wgt. / Body wgt.	Liver glucose	Liver glycogen	Plasma fructose	Plasma glucose
		g	g	%	mg/g liver		mg/100 ml plasma	
0 hours post loading								
-	-	358 ± 15[a] [1,2]	8.6 ± 0.3[a]	2.4 ± 0.50[a]	1.48 ± 0.09[a]	17.5 ± 2.1[a]	12.8 ± 1.2[a]	73 ± 6[a]
+	-	309 ± 14[b]	16.8 ± 1.3[b]	5.4 ± 0.20[b]	0.32 ± 0.04[b]	1.2 ± 0.3[b]	9.4 ± 1.3[a]	69 ± 5[a]
2 hours post loading								
-	Glu	357 ± 12[ab]	9.9 ± 0.3[a]	2.8 ± 0.05[a]	1.99 ± 0.26[ac]	36.3 ± 1.5[a]	11.2 ± 1.3[a]	117 ± 13[a]
-	Fru	385 ± 18[a]	10.0 ± 0.5[a]	2.6 ± 0.02[a]	2.14 ± 0.16[a]	34.8 ± 1.6[a]	14.1 ± 1.1[a]	78 ± 3[b]
+	Glu	327 ± 12[b]	17.6 ± 1.8[b]	5.5 ± 0.70[b]	1.37 ± 0.21[bc]	22.3 ± 3.6[b]	11.5 ± 2.8[a]	102 ± 6[a]
+	Fru	337 ± 22[ab]	18.5 ± 2.0[b]	5.9 ± 1.00[b]	1.23 ± 0.15[b]	16.6 ± 2.8[b]	13.6 ± 1.1[a]	89 ± 5[b]
4 hours post loading								
-	Glu	380 ± 15[a]	9.8 ± 0.3[a]	2.6 ± 0.05[a]	2.86 ± 0.17[a]	43.9 ± 3.4[ac]	8.6 ± 1.5[a]	75 ± 5[a]
-	Fru	385 ± 14[a]	10.8 ± 0.4[ac]	2.8 ± 0.06[a]	3.21 ± 0.15[a]	52.1 ± 3.9[a]	14.4 ± 1.7[b]	81 ± 5[a]
+	Glu	321 ± 16[b]	16.4 ± 1.3[b]	5.2 ± 0.50[b]	1.98 ± 0.22[b]	30.2 ± 2.7[bc]	9.3 ± 1.2[a]	73 ± 12[a]
+	Fru	358 ± 20[ab]	12.8 ± 0.3[bc]	3.6 ± 0.20[c]	2.23 ± 0.27[b]	37.9 ± 2.8[c]	12.9 ± 1.3[b]	76 ± 5[a]

1/ Mean ± SEM; n = 6/group at 0 hours and 9/group thereafter
2/ Values having the same superscript within a colurn in each time period are not significantly different $P<0.05$

IV.

EFFECT OF DIETARY FRUCTOSE, GLUCOSE AND LINOLEIC ACID ON LIPOGENESIS IN RATS INFECTED WITH S. TYPHIMURIUM.

The effect of high glucose and fructose diets with low and high levels of linoleic acid on carbohydrate and lipid metabolism in plasma, liver and epididymal fat pads was studied in weanling male rats infected with S. typhimurium.

In non-infected weanling rats, weight gains were similar between glucose and fructose diets. Dietary fructose significantly increased liver and spleen size regardless of dietary linoleic acid level and significantly elevated plasma fructose and hepatic fructose and glucose. Fructose with low linoleic acid significantly increased plasma triglyceride levels, with a trend toward higher but non-significant triglyceride levels in the liver and no effect on fat pads. On the other hand, with high linoleic acid the increased levels of plasma and liver triglycerides were not significant between the sugars. High levels of linoleic acid also reduced the increase of plasma triglycerides that was observed with the fructose-low linoleic acid. Surfeit dietary linoleic acid, regardless of sugar, resulted in significantly increased hepatic cholesterol synthesis.

When the weanling rats were challenged with S. typhimurium infection, fructose with low linoleic acid was a poor energy source for growth. Infected rats fed the fructose diet with low linoleic acid had lowest weight gain and highest mortality. Mortality was similar for rats fed the glucose diet with either high or low linoleic acid and the fructose diet with high linoleic acid. The glucose diet with high linoleic acid produced the best growth. The increases in liver and spleen size that resulted from fructose feeding per se in the non-infected animals were not observed in the infected rats.

In infected rats, fructose feeding significantly elevated plasma fructose levels. In general, there were no significant differences in hepatic fructose or glucose values despite a tendency toward higher levels from the fructose diet.

Disease masked the hypertriglyceridemic effect of the dietary fructose-low linoleic acid interaction. With low linoleic acid, the increase in plasma and liver triglycerides in infected rats was not significant. With high linoleic acid there was no difference in either plasma or liver triglycerides between the sugars. However, there was a significant increase in fat pad triglycerides from the dietary fructose-high linoleic acid interaction.

Compared to non-infected rats, infected animals had significant decreases in triglyceride concentrations in plasma, liver and fat pads. Disease caused significant elevations in plasma and hepatic cholesterol but diminished fat pad cholesterol and fatty acid levels. Dietary fructose had no effect on cholesterol levels in either controls or infected rats. However, compared to infected animals, non-infected rats had significant increases in plasma and liver triglycerides from fructose feeding.

Statistical analyses of these data are presented in table 6.

Table 6

Statistical summary of effect of S. typhimurium infection, dietary sugar and fat on lipid, glucose and fructose levels in plasma, liver and epididymal fat pads of weanling rats.

Treatment pair	Glucose	Fructose	Free fatty acids	Tri-glycerides	Chol-esterol	Cholesterol esters
			Plasma (mg/100 ml)			
Disease						
Control	54.6[1/]	6.9	18.1	34.0	27.8	36.8
Infected	56.2	9.5*	19.8	29.0**	33.8**	32.7*
Sugar						
Glucose	55.2	5.6	18.2	28.3	30.7	34.4
Fructose	55.6	10.8**	19.7	34.7**	31.0	35.1
Linoleic acid						
Low	54.4	8.6	17.7	32.7	31.1	34.6
High	56.3	7.8	20.2*	30.3	30.6	34.9
			Liver (mg/g x liver weight)			
Disease						
Control	12.7	1.0	24.8	5.2	15.5	10.9
Infected	13.6	1.1	28.1*	3.5**	17.9**	7.3**
Sugar						
Glucose	10.2	0.8	25.7	3.6	16.1	8.6
Fructose	16.1**	1.2**	27.2	5.1**	17.3	9.6
Linoleic acid						
Low	13.4	1.0	21.9	4.4	14.8	7.9
High	12.9	1.1	31.0**	4.3	18.7**	10.2**
			Epididymal fat pads (mg/total fat pads)			
Disease						
Control			54.5	293	33.2	
Infected			38.2*	192**	8.7**	
Sugar						
Glucose			39.5	261	23.0	
Fructose			42.2	225	18.8	
Linoleic acid						
Low			34.4	186	14.7	
High			58.3**	300**	27.2**	

1/ Mean
* Significantly different within treatment pairs by Student's t test $P<0.05$
** $P<0.01$

V.

PRELIMINARY COMPARISONS OF VOLUNTARY VS FORCED EXERCISE IN WEANLING RATS INFECTED WITH S. TYPHIMURIUM

Several preliminary studies were conducted with the objective of comparing the effects of voluntary and forced exercise on energy storage in weanling rats infected with S. typhimurium. The rats given voluntary access to the running wheels were only allowed this condition when the "forced" rats were in the wheels. Since the results were repeatable, the tabie below presents only the data from the final trial.

The presence of a good infection was confirmed by the significant increase in spleen weight (target organ for this infection) of the infected sedentary rats compared to the controls. Both voluntary and forced exercise had no effect on liver or spleen size in noninfected rats, but organ weights were significantly reduced in the infected animals due to the exercise regimen. Of interest was the lower variability in spleen size in the infected rats running voluntarily.

Both voluntary and forced exercise reduced total liver glucose and glycogen, with the latter type of exercise causing the greater reduction. Interactions with S. typhimurium further reduced reserves, indicating a double jeopardy situation.

The data confirm that forced exercise is more stressful and has greater demands on the body's energy reserves. Further, the results show that exercise in general can influence the biochemistry of infectious illness.

Table 7 . Liver and spleen weights and total liver glucose and glycogen in rats infected with S. typhimurium and subjected to 5 minutes (days 1-7) and 2 hours (day 8) of forced and voluntary exercise 8 days post infection.

Treatment	Liver wt.	Spleen wt.	Glucose	Glycogen
	g	g	mg/total liver	
Noninfected				
Nonactive (controls)	2.5	0.33	6.8	2.0
Voluntary exercise	2.3	0.44	3.9	1.2
Forced exercise	2.4	0.47	2.4	0.6
Naive/forced 1/	2.3	0.37	2.8	0.8
Infected				
Nonactive	3.8	1.37	7.6	8.4
Voluntary exercise	3.2	0.76	5.1	1.6
Forced exercise	3.0	0.72	3.6	0.8

1/ Naive/forced rats were not given access to the forced apparatus until the final 2-hour exercise period on day 8

VI.

METABOLIC RESPONSES TO FASTING AND EXERCISE STRESS IN WEANLING RATS INFECTED WITH S. TYPHIMURIUM

Experiment 1

The first study in this series was designed to determine the effect of S. typhimurium on the metabolic responses of the weanling rat to fasting during the first 24 hours post inoculation.

Fasting 24 hours resulted in a 22% decrease in body weight and a 42% reduction in liver size, of which a large part can be attributed to mobilization of glycogen stores. Glycogen levels dropped, on the average, from 29 mg/g in fed animals to 2.4 mg/g in the fasted rats. Total quantities of liver glycogen followed the same pattern, decreasing from 101 mg to 5 mg at the end of 24 hrs of fasting. Blood glucose values reflected the depletion of liver glycogen by falling from 115 mg/dl in fed animals to 89 mg/dl in the fasted rats.

Changes in carbohydrate metabolism, body weights and liver size during fasting were not influenced by the endotoxin associated with the inoculation of S. typhimurium at 24 hrs post infection. Fasting ketosis was dramatically altered by the inoculation. Both plasma betahydroxybutyrate (BOHB) and total plasma ketone bodies were repressed by the endotoxin response, with reductions of 36 and 33%, respectively. Plasma acetoacetate was not significantly altered by the endotoxin response. Other parameters altered by the inoculation were feed consumption (reduced 22% in fed infected animals). In the fed rats, body temperatures increased due to the inoculation while the fasted animals showed a decrease in body temperature following inoculation. This may be due to the double jeopardy situation involving an interaction between the two stresses. The results are summarized in table 8 below.

Table 8 . Effect of S. typhimurium 24 hrs post inoculation on fasting response in the weanling rat

Parameter	Fed -infection	Fasted -infection	Fed + infection	Fasted + infection
Body wt. (g)	64.5	50.1	61.6	48.3
Body temp. (C°)	35.9	35.5	36.1	35.2
Feed cons. (g)	9.8	-	7.7	-
Liver wt. (g)	3.4	1.9	3.3	1.9
Plasma:				
Glucose (mg/dl)	116	88	114	90
BOHB (μmole/dl)	6.4	179.9	2.6	108.7
Aceto. "	10.4	27.4	19.1	15.8
Total ketones	16.8	207.3	21.7	124.5
Liver:				
Glycogen (mg/g)	27	3	31	2
" (mg/liver)	94	6	108	4

Experiment 2

The second study in this series was designed to determine if the metabolic alterations due to S. typhimurium during fasting in the rat would also occur during the stress of forced running. Weanling rats were inoculated 6 days prior to fasting or 7 days prior to exercise. On day 7 post inoculation the fed noninfected and infected rats were forced to exercise 2 hrs at 10 rpm in running wheels; immediately after this exercise session all rats in the experiment were sacrificed.

The first observable signs of infection occurred between 48 and 72 hrs post inoculation when the infected rats showed a sharp rise in body temperature. By 96 hrs post inoculation body temperatures decreased to or below that of the controls and remained at this level. Body weights of the infected animals began to be above those of controls as the edema of infection occurred; this was noted 4 days post inoculation. At no time was feed consumption significantly influenced by the infection.

During the 2 hr exercise period, infected rats were observed to have a lower performance level and less endurance than noninfected rats; however, there was no mortality due to the forced exercise.

Fasting resulted in a 24% decrease in body weight in noninfected rats and 26% in infected animals. Organ sizes were influenced by both fasting and the infection. The disease caused a 2 and 4.6-fold increase, respectively, in liver and spleen size; fasting decreased the size of these organs 34 and 42%, respectively. The infection tended to cause an increase in epididymal fat pad size and apparently inhibited mobilization during the stress of fasting or exercise. Fasting resulted in a 44% decrease and exercise a 3.3% decrease in fat pad size in control rats, whereas in the infected animals fasting resulted in only a 19% decrease and exercise a 6.8% increase.

Metabolic changes in this study resulted primarily from fasting and exercise and were not largely influenced by the S. typhimurium. Plasma glucose was decreased 36% in fasted controls but only 20% in fasted infected rats. Blood glucose decreased 8% during exercise in controls but increased 2.6% in infected rats. Changes in blood glucose may have been influenced by not having sacrificed the animals rapidly enough post exercise, thus allowing recovery to occur. Liver glycogen was depleted by both fasting and exercise, with no apparent disease interaction. As in experiment 1, carbohydrate metabolism did not seem to be greatly influenced by the infection.

However, lipid metabolism apparently was adversely influenced by the disease. Mobilization of adipose stores seemed to be reduced, as were both fasting and exercise associated ketosis. During fasting plasma βhydroxybutyrate increased 20-fold in the controls but only 13-fold in infected rats. Exercise increased plasma BOHB 13-fold in controls but only 7.3-fold in infected animals. Total plasma ketones were influenced in a similar manner. Plasma acetoacetate was increased by both fasting and exercise 3.3 and 2.5-fold, respectively.

Hormonal alterations associated with energy metabolism were apparent in the changes in the insulin:glucagon ratio which was decreased 65% by both fasting

and exercise and correlated with the metabolic flux of energy mobilization due to these two types of stress. The infection did not appear to influence the insulin-glucagon ratio. The data are summarized in table 9 below.

Table 9. Effect of S. typhimurium on fasting and exercise induced changes in blood and liver metabolites in the weanling rat.

Treatment:	Fed:	-	-	+	+	+	+
	Exercise:	-	-	+	+	-	-
Parameter	Infection:	-	+	-	+	-	+
Body wt. (g)		92.0	89.9	117.1	118.7	121.5	121.2
Liver wt. (g)		3.2	5.2	4.3	7.4	4.7	8.0
Spleen wt. (g)		0.3	1.2	0.4	2.1	0.5	2.2
Fat pad wt. (g)		0.3	0.6	0.6	0.8	0.6	0.7
Plasma:							
BOHB (μmole/100 ml)		159	65	102	35	8	5
Glucose (mg/100 ml)		96	97	137	124	149	121
Insulin/glucagon		28	32	31	33	78	98
Liver glycogen (mg/g)		0.5	5.8	4.6	12.0	86.4	94.7

VII.

EFFECT OF EXERCISE AND DIETARY ENERGY ON ORGAN COMPOSITION OF WEANLING RATS INFECTED WITH S. TYPHIMURIUM

There were three replicated trials in this series wherein 23-day-old susceptible weanling rats were fed diets containing either 45% sucrose or 45% fructose and divided into sub-groups of controls and those infected with S. typhimurium. At 9 days post inoculation these groups were further sub-divided so that half of the rats in each group were put in running wheels and forced to run continuously for 2 hours; the remaining rats were not exercised. At the conclusion of the forced exercise session all rats were sacrificed sequentially across treatment groups. Blood, livers, spleens, hearts and epididymal fat pads were removed and quickly frozen for later analysis for lipids, glycogen and glucose. The following tables summarize the data completed at this date.

Table 10. Effect of S. typhimurium infection on body and organ weights

	Trial 1	Trial 2	Trial 3
Body weight (g)			
Control	129	119	114
Infected	121	104*	108
Liver weight (g)			
Control	5.8	5.4	5.4
Infected	7.6**	7.1**	7.6**
Liver/body weight (%)			
Control	4.5	4.6	4.7
Infected	6.3**	7.0**	7.1**
Spleen weight (g)			
Control	1.6	1.0	1.0
Infected	2.8**	2.7**	2.9**
Spleen/body weight (%)			
Control	1.2	0.8	0.9
Infected	2.3**	2.6**	2.7**

*Significantly different from controls, $P<0.05$; ** $P<0.01$

At the level administered, S. typhimurium generally reduced body weights and significantly increased the size of the spleen and liver, the target organs for this infection. Counts of numbers of organisms in control and infected spleens were performed by Dr. Solotorovsky of the Dept. of Microbiology at Rutgers and confirmed the presence of the infection and the integrity of the tissues of the control animals.

Table 11. Effect of forced exercise on triglyceride levels in plasma, liver and fat pads

	Trial 1	Trial 2	Trial 3
Plasma (mg/100 ml)			
No exercise	34	42	42
+ exercise	28	27**	30**
Liver (mg/total liver)			
No exercise	17	15	17
+ exercise	16	15	18
Fat pads (mg/total fat pads)			
No exercise	306	222	259
+ exercise	304	232	245

**Significantly different from controls, P<0.01

The single 2-hour period of exercise had no apparent effect on liver and fat pad triglyceride values even though the rats were completely exhausted at the end of the session. However, plasma triglycerides were significantly depressed.

Table 12. Effect of S. typhimurium infection on triglyceride levels in plasma, liver and fat pads

	Trial 1	Trial 2	Trial 3
Plasma (mg/100 ml)			
Control	25	31	31
Infected	37*	39	42**
Liver (mg/total liver)			
Control	15	14	16
Infected	19*	16	19
Fat pads (mg/total fat pads)			
Control	327	252	311
Infected	283	201	193**

*Significantly different from controls, P<0.05; ** P<0.01

Ratios of triglycerides among the tissues of the three trials were reasonably constant. The infection caused an elevation of triglycerides in the plasma carrier and the liver while values were depressed in the fat pad energy stores.

Table 13. . Effect of S. typhimurium infection, forced exercise and dietary energy on total liver glycogen

	Trial 1	Trial 2	Trial 3
		mg/total liver	
Infection			
Control	57	70	58
Infected	92	38	59
Exercise			
No exercise	111	99	81
+ exercise	38**	9**	36*
Dietary sugar			
Sucrose	83	48	48
Fructose	66	60	69

*Significantly different from no exercise, $P<0.05$; ** $P<0.01$

Dietary carbohydrate and the level of infectious illness administered had a variable effect on liver glycogen stores. However, in all cases the 2-hour forced exercise period significantly reduced glycogen levels. This parameter has proven to be one of the most sensitive in reflecting the energy demands of a stressor.

VIII.

EFFECT OF S. TYPHIMURIUM, FORCED EXERCISE AND DIETARY ENERGY ON CONSTITUENTS OF THE WEANLING RAT HEART AND ON MORTALITY

These experiments followed the same experimental design for length of trial, dosage of S. typhimurium, dietary composition (45% fructose or sucrose) and a single 2-hour session of forced exercise. Analyses of the hearts from these trials are in progress: lipid content, protein synthesis and enzyme reactions as well as pathological changes. The data presented herein are preliminary to the final analyses.

Table 14. Effect of S. typhimurium, forced exercise and dietary energy on heart weights

Diet:	Sucrose				Fructose			
Infection:	-	-	+	+	-	-	+	+
Exercise:	-	+	-	+	-	+	-	+
	mg/heart				mg/heart			
Trial 1	461	459	486	459	433	440	447	520
Trial 2	510	432	527	526	438	499	529	492
Trial 3	475	452	574	514	435	448	511	508

In general, 45% dietary fructose decreased heart weights compared to sucrose feeding in controls and in rats infected with S. typhimurium and/or subjected to a single 2-hour bout of forced exercise 9 days post inoculation. Dietary fructose tended to result in increased heart weights in the infected and exercised rats whereas this effect was not so apparent in the sucrose groups. When heart weights were ratioed to their respective body weights, fructose effects were even more pronounced.

In one trial the dosage of S. typhimurium was increased to supply twice the concentration of organisms of that usually used in our experiments. This resulted in 100% mortality in the groups fed 45% fructose and 50% in the sucrose groups with 6 days post inoculation.

Table 15. Effect of energy source on body weights and mortality of weanling rats infected with a high level of S. typhimurium

Diet	Exercise	Body weight	% mortality 6 days post inoc.	
			*Low level infec.	High level infec.
		g		
45% sucrose	-	122	0	50
45% sucrose	+	112	0	50
45% fructose	-	102	0	100
45% fructose	+	96	0	100

*Standard level of infection used in all other experiments

The high level of mortality in the fructose groups confirmed on-going research in our laboratories related to the adverse effects on metabolism from feeding high levels of this monosaccharide. The results confirm the potential that dietary composition may have a confounding effect on animal disease research.

IX.

COMPARISON OF EFFECT OF FORCED EXERCISE 5 and 9 DAYS POST INOCULATION ON ORGAN WEIGHTS, LIVER LIPIDS AND CARBOHYDRATES

In this trial control and S. typhimurium infected weanling rats were fed either 45% fructose or sucrose diets. Five days post inoculation, 8 rats from each of the four treatment groups were given a 2 hour session of forced exercise; the remainder of the animals were not given their forced exercise treatment until 9 days post inoculation at which time all rats were then killed and organs removed and frozen for analyses.

Table 16. Effect of forced exercise 5 and 9 days post infection on organ weights, liver lipids and carbohydrates

Diet:	Sucrose				Fructose			
Infection:	-	-	+	+	-	-	+	+
Exercise:	A[1]	B[2]	A	B	A	B	A	B
Body wt. (g)	114	122	113	97	108	113	103	102
Liver wt. (g)	4.9	5.4	7.3	6.5	5.1	5.8	7.9	7.5
Liver/body (%)	4.3	4.4	6.5	6.8	4.8	5.1	7.7	7.3
Spleen (g)	1.0	0.8	2.8	2.2	1.0	0.7	2.7	2.3
Spleen/body (%)	0.9	0.7	2.5	2.3	0.9	0.6	2.6	2.3
Fat pad wt. (g)	0.6	0.7	0.7	0.4	0.7	0.6	0.7	0.5
Fat pad/body (%)	0.5	0.6	0.6	0.4	0.7	0.5	0.7	0.5
Total liver:								
Fructose	0.5	1.2	0.8	1.1	0.3	1.3	0.8	1.2
Glucose	4.8	18.8	10.5	18.3	3.6	23.0	10.0	19.1
Glycogen	23.2	172.9	57.2	171.3	28.4	209.4	34.6	155.7
Free fatty acids	19.8	25.7	26.6	28.8	21.2	27.1	29.1	36.6
Triglycerides	19.4	13.1	19.9	14.5	13.6	14.8	20.1	16.8
Monoglycerides	15.2	21.3	22.8	26.6	15.8	20.0	26.6	28.9
Diglycerides	11.9	13.2	14.5	16.7	9.5	14.4	14.3	18.2
Cholesterol	14.9	17.3	24.1	21.1	15.4	17.4	26.9	24.8
Chol. esters	10.9	9.3	9.5	7.0	10.5	8.4	9.7	8.2

1/ Exercised 2 hours 9 days post inoculation; sacrificed 9 days post inoculation
2/ " " 5 " " ; " 9 " "

When all treatment are considered, the overall effect from disease or exercise, whether there was an increase or decrease, was for the values to be slightly more or less in the fructose groups than in the sucrose groups. An interesting phenomenon occurred in the effects of exercise 5 and 9 days post inoculation - free fatty acids were higher and triglycerides lower when the exercise was applied 5 days rather than 9 days post inoculation. In general, the magnitude of changes of the various parameters in reaction to time of exercise in the infectious process would indicate that the stress levels applied minimal. Future experiments will investigate higher involvement levels for the infection and more than a single forced exercise session.

Table 17 . Effect of forced exercise 5 and 9 days post infection on protein synthesis in the rat heart

Diet:	Sucrose				Fructose			
Infection:	-	-	+	+	-	-	+	+
Exercise:	A[1]	B[2]	A	B	A	B	A	B
	% change from respective controls							
RNA (total heart)	-5	+15	+15	+6	+3	+13	+17	+35
Protein "	-17	+ 5	+25	+34	+4	+13	+33	+58
Cathepsin D	+19	+ 8	+56	+17	+22	+53	+48	+90

1/ Exercised 2 hours 9 days post inoculation; sacrificed 9 days post inoculation
2/ " " 5 " " ; " 9 " "

Infection and infection x exercise increased protein synthesis. Greatest increase occurred when sucrose was the major source of energy. Forced exercise on day 5 with sacrifice on day 9 appeared to result in greater magnitudes of changes than when animals were sacrificed immediately following the exercise session. These phenomena suggest changes in experimental designs.

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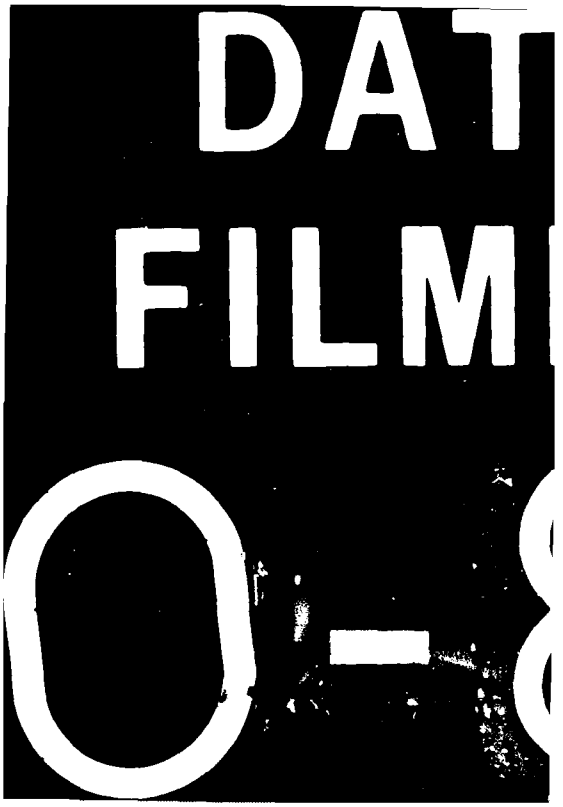
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